Eur. J. Clin. Chem. Clin. Biochem.
Vol. 32, 1994, pp. 511–514

Prognostic Significance of Autoantibodies to Laminin in the Sera of Breast Cancer Patients: A Preliminary Report[1])

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(Received November 29, 1993/April 11, 1994)

Summary: Interactions between cancer cells and laminin play an important role in the metastatic cascade. The aim of this retrospective study was to evaluate the presence and the prognostic significance of autoantibodies to laminin in sera of patients with breast cancer. Our study population consisted of 71 high-risk primary breast cancer patients (median follow-up, 6 years). IgG, IgA and IgM were analysed at the time of surgery and before any treatment was given. Concentrations were measured by enzyme immunoassay with a highly purified antigen. IgG, IgA and IgM anti-laminin median values were, respectively 117%, 130% and 97% of normal absorbance. Higher concentrations of IgM to laminin were found in pre-menopausal patients as compared with post-menopausal patients ($p < 0.001$). Patients with high concentrations of IgG to laminin had lower disease-free ($p = 0.003$) and overall survival rates ($p = 0.003$). In breast cancer, assay of IgG to laminin could thus provide a cheap and easy prognostic index available for all patients.

Introduction

Autoantibodies of various specificities have been identified in the sera of patients suffering from neoplasic diseases (1–5). In breast cancer, until now, only anti-nuclear and anti-smooth muscle autoantibodies have been correlated with the clinical course of the disease (6).

Cancer invasiveness is determined by basement membrane fragmentation. Interactions between cancer cells and laminin, the main glycoprotein of basement membranes, play an important role in the metastatic cascade (7). In addition, laminin-derived peptides stimulate angiogenesis and tumour growth (8).

The aim of this retrospective study was to evaluate the presence and the prognostic significance of autoantibodies to laminin in sera of 71 high-risk primary breast cancer patients. The association of IgG, IgA and IgM anti-laminin with classical prognostic factors, disease-free and overall survival was studied to determine their possible clinical usefulness.

Materials and Methods

Patients

Our study population consisted of 71 high-risk primary breast cancer patients (mean age = 51 years; range = 33–67 years) who were treated by the same clinical team between 1984 and 1988 (tab. 1). The median follow-up was 6 years (maximum follow-up, 8 years). All patients had undergone partial mastectomy with axillary lymph node clearance and post-operative irradiation as part of the locoregional treatment. Patients received adjuvant chemotherapy (n = 34) or hormone therapy (n = 37). At the time of analysis, 27 patients had distant metastases and 20 had died of cancer.

Macroscopic tumour size was defined as the largest tumour diameter measured by the pathologist. Axillary node involvement was determined by pathological examination of at least 10 nodes per

[1] This work was suppoted by grant No. 6257 from Association pour la Recherche sur le Cancer.

Tab. 1 Characteristics of patients

Condition	Number of probands	
	absolute	relative (%)
Age (years)		
< 50	28	39.4
≥ 50	43	60.6
Menopausal status		
Pre-	31	43.7
Post-	40	56.3
Size of tumour (cm)		
< 2	16	22.5
[2–5]	44	62.0
≥ 5	6	8.5
unknown	5	7.0
Number of axillary nodes involved		
< 3	13	18.3
≥ 3	58	81.7
*Histological grade (SBR)**		
I	6	8.5
II	25	35.2
III	35	49.3
unknown	5	7.0
Oestrogen receptors		
< 10 nmol/kg protein	23	32.4
≥ 10 nmol/kg protein	46	64.8
unknown	2	2.8
Progesterone receptors		
< 10 nmol/kg protein	33	46.5
≥ 10 nmol/kg protein	36	50.7
unknown	2	2.8

* SBR: *Scarff, Bloom, Richardson* classification (9)

patient. The histological grade was determined using the method of *Scarff, Bloom* and *Richardson* as decribed in l. c. (9).

Assay of steroid receptors

Oestrogen and progesterone receptors were assayed using the dextran-coated charcoal method (cut-off, 10 nmol/kg protein). Quality control was assured by frequent testing with both internal controls and European Organization for Research and Treatment of Cancer (EORTC) standards (10).

Assay of autoantibodies to laminin

The sera were collected at the time of surgery and prior to therapy and stored at −20 °C. Concentrations of autoantibodies to laminin were measured by enzyme immunoassay as previously described (11). Laminin antigen was highly purified from mouse *Engelbreth-Holm-Swarm* sarcoma extracts by anion exchange chromatography and gel filtration as previously described (12).

Calculation

Results of autoantibody assay are expressed as relative absorbance:

$$\frac{\text{absorbance of tested serum}}{\text{absorbance of pool of 80 normal sera}} \cdot 100\ (\%).$$

Statistics

Relationships between autoantibody concentrations were analysed using the *Spearman* rank test; differences in the distribution among patients subgroups were studied using the *Mann-Whitney* U test. Survival rates, calculated according to the *Kaplan-Meier* method were compared using the log rank test. The cut-off value for autoantibodies to laminin was established in order to achieve the highest chi-square value (χ^2) resulting from log rank tests.

Results

Distribution of autoantibodies to laminin

IgG, IgA and IgM anti-laminin concentrations (relative absorbance values) were, respectively: means ± SD: 133 ± 79%, 159 ± 93% and 124 ± 90%; medians: 117%, 130% and 97% (fig. 1). The concentrations of IgG and IgA autoantibodies to laminin were highly correlated ($p < 0.001$). IgM concentrations were more weakly correlated to IgG ($p = 0.002$) and to IgA concentrations ($p = 0.02$).

Association between autoantibodies to laminin and tumour characteristics

Higher concentrations of IgM to laminin were found in pre-menopausal patients, compared with post-menopausal patients (median 138 vs 85% relative absorbance) ($p < 0.001$). No other significant correlation was found between autoantibodies to laminin and tumour size, axillary node involvement, histological grade or steroid receptors.

Associations between autoantibodies to laminin and other factors with (disease-free) survival

Menopausal status, tumour grade, oestrogen receptor and progesterone receptor status showed no significant

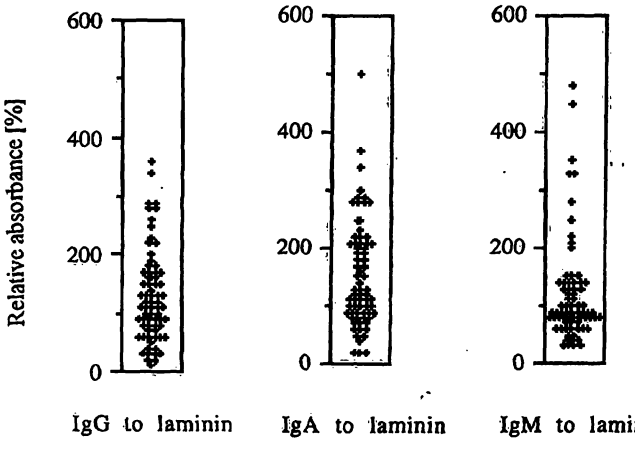
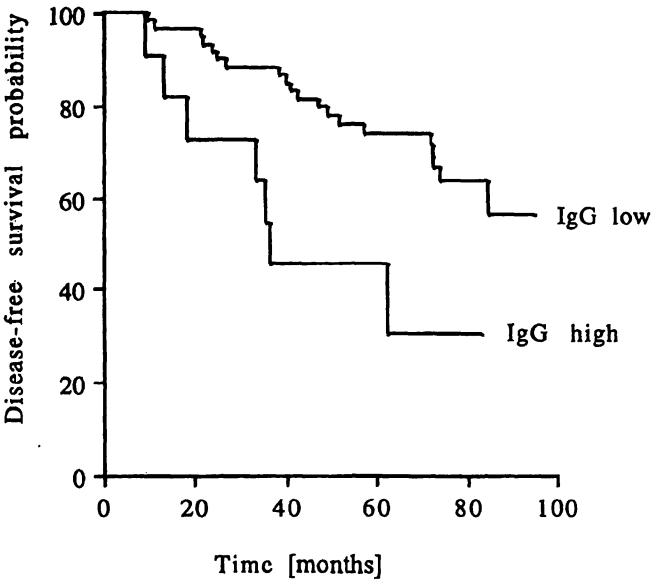


Fig. 1 Distribution of antibodies to laminin in the sera of 71 breast cancer patients. Concentrations are expressed as relative absorbance (comparison with a pool of 80 normal sera), see section "Calculation".

association with disease-free and overall survival. Larger tumour size was associated with reduced disease-free ($p = 0.0003$) and overall survival ($p = 0.0002$). The number of axillary lymph nodes involved was positively associated with both a shorter disease-free ($p = 0.02$) and overall survival ($p = 0.01$).

Analysis of a possible correlation between autoantibodies and outcome showed that patients with high concentrations of IgG to laminin had lower survival rates. The cut-off value was established to achieve the best clinical correlation. The highest χ^2 was observed at a cut-off of 210% relative absorbance (disease-free survival, $\chi^2 = 8.96$, $p = 0.003$; overall survival, $\chi^2 = 8.57$, $p = 0.003$) (fig. 2). No significant association could be found between prognosis and the concentrations of IgA or IgM to laminin.



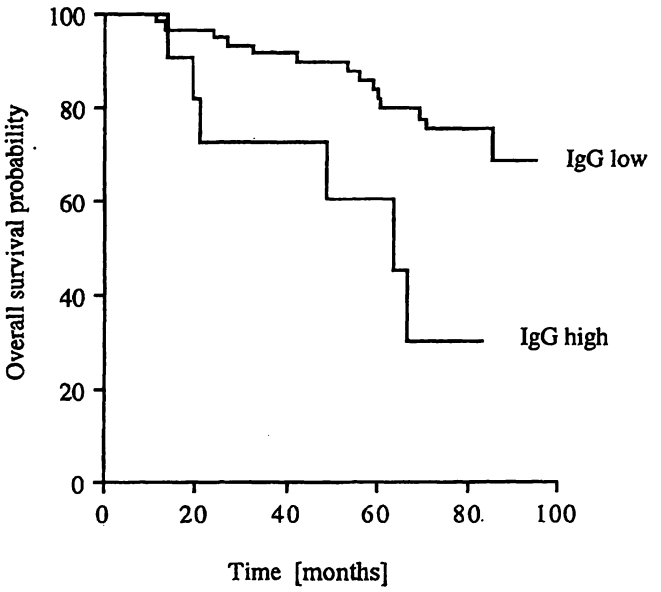


Fig. 2 Disease-free and overall survival of 71 breast cancer patients according to serum concentrations of IgG antibody to laminin. Concentrations are expressed as absorption (comparison with a pool of 80 normal sera), see section "Calculation"; low: < 210% (n = 60); high: ≥ 210% (n = 11).

Discussion

We demonstrate here a high incidence of antibodies to laminin to the sera of breast cancer patients at the time of diagnosis and before any therapeutic intervention. Dysregulation of the immune system and accelerated lysis of neoplastic tissues have been proposed as stimuli for autoantibody production in patients with malignancies (13).

Laminin has been implicated in cell adhesion and migration. In previous studies, it was suggested that intratumoural laminin distribution (14), laminin receptors (15), serum (16) and urinary (17) laminin fragments may have a prognostic value in breast cancer. We found a statistically significant association between IgG antibodies to laminin and poor prognosis. Although this association needs to be confirmed in prospective clinical trials including more patients, assay of IgG to laminin would provide a cheap and easy test available for all patients.

Several proteases, including principally the cathepsin D (18) and the urokinase plasminogen activator (19), have been associated with an increased risk of developing metastasis in breast cancer. Intratumoural proteases only reflect tumour aggressiveness at the time of surgery, whereas autoantibodies to laminin can be interpreted as the cumulative result of both tumour aggressiveness and the time course of the disease.

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